# Correlation Between High-Resolution Computed Tomography, Neutrophilic Inflammation, Air-way Obstruction and Serum Interleukin-8 Levels in Chronic Obstructive Pulmonary Disease

# Kronik Obstrüktif Akciğer Hastalığında Nötrofilik İnflamasyon, Hava Yolu Obstrüksiyonu ve Serum İnterlökin-8 Düzeyleri İle Yüksek Rezolüsyonlu Bilgisayarlı Tomografi Bulguları Arasındaki Korelasyon

KOAH'da İnflamasyon / Inflammation in COPD

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**Özet**

Amaç: Bu çalışmada stabil KOAH'lı olgularda nötrofilik inflamasyon, hava yolu obstrüksiyonu ve serum interlökin-8(IL-8) düzeylerinin yüksek rezolüsyonlu bilgisayarlı tomografi ile ilişkisi araştırıldı. Gereç ve Yöntem: Şubat 2005-Ocak 2006 tarihleri arasında 23 Evre I, 15 Evre II ve 12 Evre III-IV stabil KOAH'lı hasta çalışmaya alındı. 10 sağlıklı sigara içmeyen, 20 sağlıklı sigara içen olgu çalışmaya kontrol grubu olarak dahil edildi. Solunum fonksiyon testleri yapıldı, balgam indüksiyonu ile eş zamanlı kan örneği alındı. Yüksek rezolüsyonlu bilgisayarlı tomografi çekildi ve görsel skorlama yöntemi ile değerlendirildi. Bulgular: Sigara içen gruplarda KOAH evresi arttıkça FEV1 değeri düşmekte toplam akciğer skoru (TAS) artmaktaydı. KOAH evresi artışı ile paralel olarak nötrofil sayısında artma, makrofaj sayısında ise azalma izlendi. KOAH evresi arttıkça IL-8 düzeyi artmakta idi. TAS ile FEV1 arasında ve nötrofil sayısı ile FEV1 arasında negatif korelasyon izlendi(p<0,001). Nötrofil sayısı ile TAS ve IL-8 ile TAS arasında pozitif korelasyon izlenirken IL-8 ile FEV1 arasında negatif korelasyon saptandı(p<0.001). Tartışma: KOAH'taki inflamasyonun derecesini ve ağırlığını belirlemede IL-8, indükte balgam incelemesi; fonksiyonel ve anatomik değerlendirme ve evrelemede erken dönemde YRBT değerlendirmesinin kullanılabileceği sonucuna varıldı.

**Anahtar Kelimeler**

KOAH; YRBT; İndükte Balgam; IL-8

**Abstract**

Aim: In this study, the correlation between high-resolution computed tomography (HRCT) and neutrophilic inflammation, air-way obstruction and serum IL-8 levels was examined in subjects with stable COPD. Material and Method: 23 stage I, 15 Stage II and 12 Stage III–IV patients with COPD were included in the study. 10 healthy nonsmoking and 20 smoking participants were additionally included to comprise the control group. Pulmonary function tests, sputum induction and blood collection were performed and HRCT assessed using the visual scoring method. Results: We observed that the forced expiratory volume per second (FEV1) decreased and the total lung score (TLS) increased in smoking groups. An elevated number of neutrophils was observed in parallel with increasing stages of COPD, whilst the number of macrophages decreased in parallel. As the stage of COPD increased, the levels of IL-8 increased. A negative correlation between TLS and FEV1 and the number of neutrophils and FEV1 was observed among all subjects (p<0.001). A positive correlation was determined between the number of neutrophils and TLS, and IL-8 and TLS, whilst a negative correlation was observed between IL-8 and FEV1 (p<0.001). Discussion: When determining the degree and severity of inflammation in COPD, IL-8 analysis of induced sputum and both functional and anatomic assessment and staging assessment of HRCT in the early stage, can be effectively utilised.

**Keywords**

COPD; HRCT; Induced Sputum; IL-8

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## Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity worldwide. Chronic airflow obstruction, the typical characteristic of COPD, is generally progressive and is associated with an abnormal inflammatory response of the lung to noxious particles, gases and in particular, cigarette smoke [1–3]. Numerous cells and mediators of inflammation exist in COPD and an elevation in neutrophils, macrophages and T lymphocytes is typically observed.

Airway and alveolar epithelial cells are important sources of the inflammatory mediators. Pro-inflammatory mediators such as eicosanoids, cytokines and adhesion molecules are released following synthesis from the bronchial epithelial cells of healthy subjects inhaling noxious gasses [4]. Studies have known that the production of inflammatory mediators, particularly interleukin-8 (IL-8), leukotriene B4 (LTB4) and tumor necrosis factor-alpha (TNF) increase in COPD and that the neutrophils are the main source of these mediators. The production of these mediators has been utilised in the assessment of the severity of airway inflammation. In addition, induced sputum, a material obtained from the lower respiratory tract, can be used to assess the severity of airway inflammation [4; 5].

The prevalence and anatomical distribution of parenchymal damage can be easily assessed using high-resolution computed tomography (HRCT). Focal or diffuse air trapping can be determined following HRCT examination performed during expiration in subjects with airway obstruction. Consequently, inspiratory and expiratory HRCT plays an important role in the follow-up of the population at risk of developing obstructive disease [6].

In the present study, the correlation between neutrophilic inflammation, airway obstruction and serum levels of IL-8 and HRCT findings was investigated in subjects with COPD. The current study combined these parameters and assessed their usefulness in the role of early diagnosis.

## Material and Method

*Patients (inclusion and exclusion criteria)*

Fifty patients receiving a diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Cough Consensus Panel Reports [1] in Atatürk Chest Diseases and Chest Surgery Education and Research Hospital were included in the study. The participants were clinically stable during the last 8 weeks and were using beta agonists or anticholinergics and did not use antibiotics or anti-inflammatory drugs such as inhaled/oral corticosteroids. Ten healthy nonsmoking and 20 smoking participants were also included to comprise the control group. The study was performed with the consent of the hospital ethics committee.

Subjects were excluded from the study if they did not agree to participate or did not have a COPD diagnosis; they were disqualified if they had atopy or were younger than 18 or older than 75 years of age; subjects with viral and bacterial infections, other inflammatory diseases, autoimmune diseases and malignancies that could affect IL-8 levels were also excluded. Lung function tests, induced sputum and serum IL-8 measurements.

The value of the forced expiratory volume per second (FEV1), the forced vital capacity (FVC), the ratio of residual volume to total lung capacity (RV/TLC), the diffusing capacity of the lung for carbon monoxide (DLCO) and the DLCO/alveoli ventilation (DLCO/VA) values were obtained to determine airflow impairment using a Sensormedics V max 229 (Sensormedics, Yorba Linda, CA) series flow-sensitive spirometer in the Pulmonary Function Test Laboratory at our hospital. The diffusion capacity was measured using the single-breath method and the values were corrected according to age, height and weight. Patients were classified according to GOLD 2006 criteria as follows (with airflow limitation): FEV1/FVC < 70%; Stage I Mild COPD: FEV1 ≥ 80% predicted; Stage II Moderate COPD: 50% ≤ FEV1 < 80% predicted, Stage III Severe COPD: 30% ≤ FEV1 < 50%; or Stage IV Very Severe COPD: FEV1 < 50% predicted + the presence of respiratory failure.

Sputum induction was performed on the same day in the morning as previously described [7–9].

The sputum was separated macroscopically from saliva, and 0.1 mg/ml (0.1%) dithiothreitol (DTT) was added to bind glycoprotein fibres and unfold disulphide bonds, ensuring the gelous form of sputum achieving mucolysis and ensuring homogenisation. The sputum specimen for cell analysis was added in equal amounts. The sputum was incubated at 37˚C for 20 min, vortexed every 5 min and centrifuged at 2000 g for 10 min. The sediment was washed in D-PBS and the total number of cells counted using a Neubauer haemocytometer (Paul Marienfeld GmbH & Co., Königshofen, Germany). Then $1 \times 10^6$ cells/ml were centrifuged at 450 g for 6 min, air-dried, stained with Wright's stain and the cell distribution assessed (400 cells) [10; 11].

Then 5 ml of blood was collected from the subjects simultaneously with the sputum induction. The samples were stored at –80˚C prior to the assessment of IL-8 serum levels using an ELISA kit (BioSource International Inc., Camarillo, CA, USA). Values of IL-8 were expressed in pg/ml.

*HRCT*

The inspiratory and expiratory HRCT sections were obtained and HRCT analyses performed on two occasions, following the performance of both deep inspirium and expirium. Each HRCT was scanned using a Pratico Spiral device (Hitachi, Tokyo, Japan) with a 10 mm table movement and 1 mm slice thickness (512 × 512 matrix size) without contrast material administration in the axial plane. HRCT Images, with a window width of 1200 Hounsfield units (HU) and a window level of 700, were obtained. HRCT scans were acquired at three levels: 3 cm below the apex of the lung, at the hilus and 3 cm above the diaphragm. Six scan areas were used. The visual score method was utilised for the assessment of air trapping in the HRCT analyses. In this method, HRCT scans were estimated at each lung using a 5-point scale: 0 (no air-trapping visible), 1 (1–25% of the cross-sectional area of lung affected), 2 (2–50% affected), 3 (51–75% affected) and 4 (76–100% affected) [12].

The total lung score was equal to the sum of the total points of six sites of the cross-sectional areas. Visual analysis was performed without obtaining data from clinical situations, pulmonary function test (PFT) values and lung graphy. As the correlation between the observers and repeating observations are known in relation to the visual scoring of air-trapping, cross sections were assessed by two different radiologists.

### *Statistical analyses*

Statistical analyses were performed using the SPSS 11.5 for Windows package programme (SPSS Inc., Chicago, IL, USA). An analysis of distribution of constant variance for normality was assessed using the Shapiro–Wilks' test. Descriptive statistical analyses for gender were performed in the number and percentage of subjects, expressed as the mean ± SD for age and pack-years, and other clinical measurements were reported in the median (interquartile range). The significance of age with respect to age averages between the groups was examined using one-way ANOVA and Tukey's post hoc test. The Kruskal–Wallis test was used to identify the significance of differences between median values of clinical measurements. When the comparison between groups indicated a significant result, nonparametric multiple comparison tests were performed to identify the group responsible for the differences. Nominal variables were investigated with Pearson's chi-square and Fisher's exact tests. A p-value less than 0.05 was considered as statistically significant.

## Results

Of the 80 subjects included in the study, 58 were male (72.5%) and 22 were female (27.5%). The male dominance in the group was considered to be due to male dominance in cigarette smoking in our country. The age of the subjects ranged from 47 to 65 years with a mean of 54.10 years. Ten of the subjects (12.5%) were healthy nonsmokers (Group 1), 20 (25%) were healthy smokers (Group 2), 23 had (28.75%) Stage I COPD (Group 3), 15 (18.75%) had Stage II COPD (Group 4) and 12 (15%) had Stage III–VI COPD (Group 5). The demographic data and clinical measurements of the subjects are shown tables 1 and 2. As the stage of COPD increased, the FEV1 values decreased in the cigarette smoking group. Stages III–IV displayed the lowest FEV1 values (37.0%, $p < 0.001$). The total lung score (TLS) increased as the COPD stage increased. In Stages II and III–IV COPD TLS values were significantly higher ($p < 0.001$). No significant differences were observed between two groups ($p > 0.05$). No significant differences were also observed between the total cell distributions among all groups ($p > 0.05$). Parallel to the increase in COPD stage, an increase in the number of neutrophils was evident; however, the number of macrophages decreased as the stage of COPD increased. As the COPD stage increased, increased IL-8 levels were also detected. A strong negative correlation was observed between TLS and FEV1 among all groups ($p < 0.001$) (Figure 1). A strong negative correlation between the number of neutrophils and FEV1 was also evident ($p < 0.001$) (Figure 2).

A positive correlation was determined between the number of neutrophils and TLS values ($p < 0.001$) (Figure 3). A positive correlation between the IL-8 and TLS and a negative correlation between IL-8 and FEV1 were determined ($p < 0.001$) (figures 4 and 5).

Table 1. Demographic Datas and Correlations

| | Non-smoker control Group 1 (n=10) | Smoker control Group 2 (n=20) | Stage I COPD Group 3 (n=23) | Stage II COPD Group 4 (n=15) | Stage III-IV COPD Group 5 (n=12) | p |
|---|---|---|---|---|---|---|
| Age (years) | 47.2±4.2 | 45.7±2.2 | 52.9±7.4 | 63.5±12.5 | 64.3±7.0 | <0.001 |
| Male gender | 2 (%20) | 13 (%65) | 18 (%78.3) | 13 (%86.7) | 12 (%100) | <0.001 |
| Cigarette (Pack/years) | - | 19.5±8.1 | 22.5±11.5 | 41.1±25.0 | 50.1±29.9 | <0.001 |

Table 2. Clinical Measurements

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | p |
|---|---|---|---|---|---|---|
| FEV1 | 102.5 (13.75) | 96.0 (12.25) | 86.0 (13.00) | 65.0 (19.00) | 37.0 (12.25) | <0.001 |
| FVC | 104.0 (13.50) | 93.0 (11.25) | 96.0 (12.50) | 83.0 (10.00) | 66.5 (28.50) | <0.001 |
| FEV1/FVC | 85.0 (4.50) | 84.5 (4.00) | 77.0 (16.50) | 60.0 (10.00) | 56.5 (14.75) | <0.001 |
| RV/TLC | 38.5 (6.50) | 31.0 (4.00) | 41.0 (9.50) | 48.0 (15.00) | 56.0 (8.25) | <0.001 |
| DLCO | 84.0 (5.75) | 80.0 (7.50) | 72.0 (9.50) | 74.0 (18.00) | 67.0 (10.50) | <0.001 |
| DLCO/VA | 82.0 (6.25) | 78.0 (9.75) | 71.0 (9.00) | 76.0 (18.00) | 71.5 (14.00) | <0.001 |
| TLS | 2.5 (1.50) | 4.0 (1.75) | 7.0 (6.00) | 17.0 (9.00) | 20.5 (5.75) | <0.001 |
| Total cell count (cells x$10^6$) | 2.0 (2.21) | 4.4 (11.57) | 2.9 (2.81) | 2.8 (3.00) | 5.2 (7.56) | 0.039 |
| Neutrophils | 32.5 (23.50) | 45.5 (31.75) | 48.0 (31.50) | 64.0 (13.00) | 69.5 (20.00) | <0.001 |
| Macrophages | 65.0 (20.00) | 36.5 (30.50) | 49.0 (28.00) | 35.0 (19.00) | 27.5 (19.50) | <0.001 |
| IL-8 | 117.5 (34.50) | 265.0 (62.50) | 710.0 (118.00) | 992.0 (50.00) | 1121.0 (201.00) | <0.001 |

$FEV_1$: Forced expiratory volume per second as liter
FVC: Forced vital capacity as liter
RV/TLC: The ratio of residual volume to total lung capacity
DLCO: Diffusing capacity of the lung for carbon monoxide as mL/seconds/mmHg
DLCO/VA: Diffusing capacity of the lung for carbon monoxide/ alveoli ventilation
TLS: Total lung score
IL-8: Interleukin-8

## Discussion

The GOLD 2006 criteria for COPD list two important new dimensions. First, COPD is a disease developing with systemic effects, and second, it is preventable and treatable. Since COPD is understood to be a global health problem causing an increased economic and social burden, investigations on the early diagnosis and treatment of COPD have accelerated [1].

The diagnosis of COPD relies on both functional and radiographic criteria. Pulmonary function tests are easily applicable, noninvasive and cheap methods in the diagnosis, follow-up and the assessment of disability. However, PFT may become insufficient, particularly during the early stage of the disease, when the functional loss is insignificant. The most effective diagnostic method at this stage is to reveal air-trapping based on the performance of quantitative HRCT measurements of lung tissue density [13]. Lucidarme and colleagues deduced that air-trapping suggests airway obstruction in subjects with clinically suspected COPD, but the PFTs are normal in these subjects and the air-trapping scores calculated on the expiratory HRCT were correlated with the degree of airway obstruction measured by PFT [6]. HRCT has succeeded in the detection of morphologically low-level regions of emphysaema that cannot be detected by PFT and chest radiography, with high sensitivity [13].

HRCT findings have been compared with PFT values in numerous studies. A significant

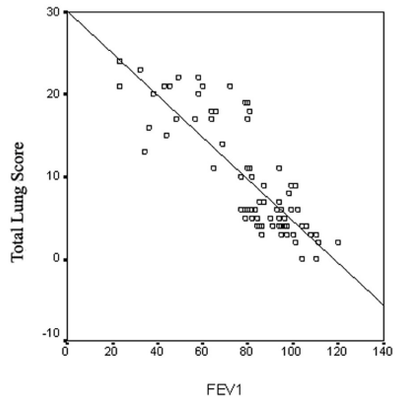


Figure 1. Relationship between TLS and FEV1

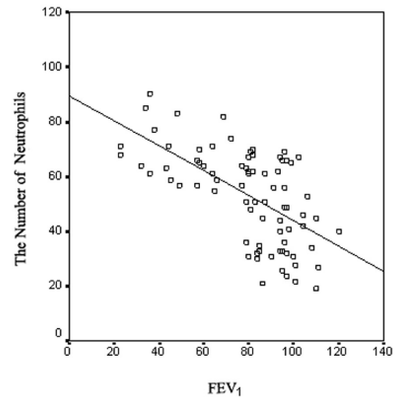


Figure 2. Relationship between the number of neutrophils and FEV1

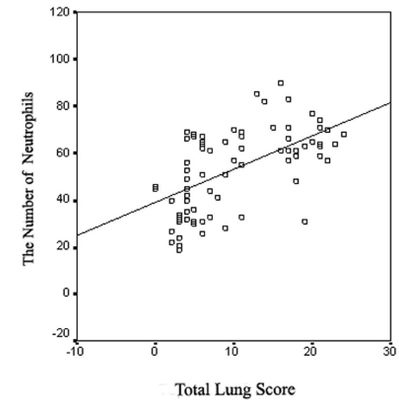


Figure 3. Relationship between the number of neutrophils and TLS

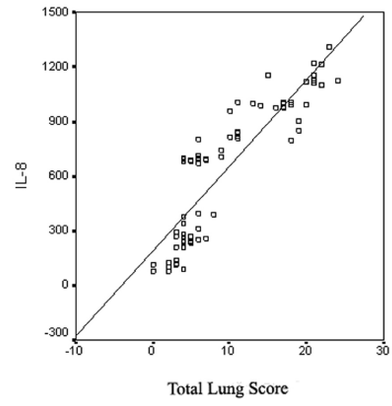


Figure 4. Relationship between IL-8 and TLS

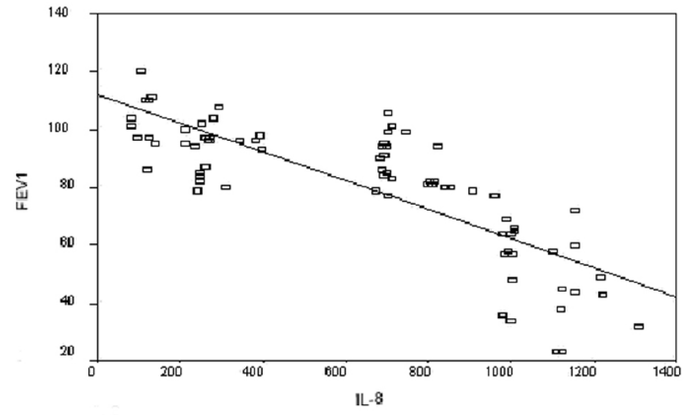


Figure 5. Relationship between IL-8 and FEV1

correlation between decreased attenuation (one of the most common HRCT findings) and FEV1, $FEF_{25-75}$ and RV has been reported [14]. Sakai et al [15] used the visual scoring method (depending on the prevalence and severity of emphysaema) to determine a strong correlation between visual scoring values and pulmonary function test values. They determined the strongest correlation using the percentage of FEV1, FEV1/FVC consistent with other studies [16]. Additionally we detected a strong negative correlation between TLS and FEV1.

Focal lucent areas at expiratory sections can be observed in some normal subjects whereby an increase in lung density normally observed in these regions cannot be observed due to air-trapping. These images appear most typically in the superior segments of the lower lobes, anterior middle lobes or the lingula. Additionally, technically uncontrollable factors such as patient mismatch and air pollution exist [17]. Özer and colleagues [18] reported that in patients with Behçet's disease, a small airway disease at an early stage could be diagnosed through detecting air-trapping areas, whilst nonsmoking patients were asymptomatic or had normal PFT values. In the current study, TLS was found to be 2.5 (1.50) in nonsmoking subjects, which may be associated with these effects. We considered that, if these effects were excluded, the early detection of air-trapping on HRCT in nonsmoking and smoking subjects without COPD could be utilised for the treatment and prognosis prior to the initiation of the disease.

Induced sputum has been used in the diagnosis, treatment and follow-up of COPD treatment and asthma, since it was determined as useful in the investigation of airway inflammation. In the current study, we observed no side effects in patients during the test and no occurrences of a FEV1 decrease of more than 20% during the follow-up of the pulmonary function test. In addition, no patients discontinued the test consistent with the low prevalence of side effects during sputum induction as previously reported. In this regard, Khajotia and coworkers [19] observed no side effects among 25 patients, but two patients were not able to co-operate completely with the process. In conclusion, sputum can be safely induced in patients with COPD.

IL-8 is a chemoattractant and an activator of cytokines for neutrophils. IL-8 has been shown to be a useful marker when determining the severity of airway inflammation. In previous studies, the levels of IL-8 were reported to increase in the induced sputum of the bronchoalveolar lavage fluid and in the serum [20]. According the format of the study we assessed serum IL-8 level but induced sputum IL-8 levels could be worked.

Perng and colleagues [21] determined that the number of sputum neutrophils was negatively correlated with FEV1 values in subjects with stable COPD. IL-8 was determined to be correlated with the number of sputum neutrophils, and the numbers of sputum neutrophils and IL-8 were subsequently deduced to be associated with pulmonary functional impairment. In the present study, an increase in the level of IL-8 parallel to the increase in number of neutrophils was detected and a strong negative correlation between the number of neutrophils and FEV1 values was observed. O'Donnell and colleagues [22] examined the relationship between IL-8 and TLS and reported that TLS might

increase in smoking subjects at early stages when airway obstruction does not develop. This study provided a positive correlation between IL-8 and TLS. Consistently, in the current study, a positive correlation between IL-8 and TLS was observed. Parallel to these results, a positive correlation between neutrophils and TLS and a negative correlation between FEV1 and IL-8 was determined.

The dominant inflammatory cells in the airways of subjects with COPD are neutrophils. In studies performed by Peleman and coworkers a high number of cells in the sputum were evident in COPD when compared with those of the healthy adult group. The cell distribution in the induced sputum of patients was measured as 22.5% neutrophils and 74.0% macrophages in the control group and 74.9% neutrophils and 20.9% macrophages in the COPD group. These studies concluded that the presence of a high number of neutrophils was present in the sputum of patients of COPD, whilst a dominance of macrophages in the sputum of healthy adults occurs. Subsequently, it was deduced that the induced sputum was a reliable method for the detection of inflammatory cells in COPD [23]. Rutgers and coworkers [24] calculated the cell count to be 0.4–29.3 (mean 2.6) × $10^6$ in an induced sputum assessment performed on subjects with COPD. We observed that in the nonsmoking control group, of the total number of cells (2.5 × $10^6$), 33.4% were neutrophils whilst 63.2% were macrophages, but in the smoking control group, the total number of cells was 7.9 × $10^6$ and 48.7% were neutrophils and 43.8% macrophages. In subjects with COPD, we found 3.83 × $10^6$ cells of which 58.19% were neutrophils and 35.77% macrophages. Thus, no significant differences among all subjects regarding the total number of cells were evident ($p > 0.05$).

In conclusion, the high levels of IL-8 in the serum of subjects with COPD are an indicator of inflammation in the lungs and a systemic influence, in addition to the related damage. Thus, if PFT values were normal in nonsmoking subjects or healthy smoking subjects with no COPD development, air-trapping revealed by HRCT may be diagnosed early, dictating the treatment and prognosis. Consequently, in determining the degree and severity of inflammation in COPD, the IL-8 analysis of induced sputum, with functional and anatomic and staging assessment of HRCT, can be used in the early stages of disease

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